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# Soil Enzymatic Activity under Different INM Practices in Rice-Rice Cropping System

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**A B S T R A C T**

Studies were conducted to understand the influence of integrated nutrient management on soil enzymatic activity under rice-rice cropping system since 1988 at Agricultural College Farm, Rajendranagar, Hyderabad. The enzyme activities *viz.*, urease (55.8, 54.2 µg of $NH_4^+$-N $g^{-1}$ soil $2h^{-1}$), dehydrogenase (488.23, 364.3 µg of TPF $g^{-1}$ soil $day^{-1}$) and acid (79.28, 70.50 µg p-nitrophenol $g^{-1}$ soil $h^{-1}$) and alkaline phosphatase (149.70, 129.53 µg p-nitrophenol $g^{-1}$ soil $h^{-1}$) were significantly higher in treatment $T_3$ (50 % RD of NPK + 50 % N through FYM) in both *kharif* and *rabi* seasons. The enzyme activity of soils, which is governed by microbial population is also significantly higher in INM treatments. Our results demonstrate that soil enzymatic activity acted as a useful indicator of soil fertility dynamics. Enzymatic activities were positively and significantly correlated with content of organic carbon.

## Introduction

The role that microbial activity plays in ecosystem processes is significant because approximately 80% to 90% of soil processes are mediated by microorganisms (Nannipieri and Badalucco, 2003). Soil microbial population are the driving force behind regulating soil processes such as organic matter decomposition and nutrient cycling, it is imperative to have a better understanding of the factors that regulate its size, activity, and structure (Masto *et al.*, 2006). Soils containing a high microbial diversity are characteristic of a healthy soil-plant relationship, whereas those with low microbial diversity are characterized as an unhealthy soil that often hardly responds to environmental changes (Tejada *et al.*, 2011).

Phosphatases find widely in bacteria to mammals, and indicate their importance in

fundamental biochemical processes. The term phosphatase in soil is used to describe a group of enzymes that are responsible for the hydrolytic cleavage of a variety of ester-phosphate bonds of organic phosphates and anhydrides of orthophosphoric acid ($H_3PO_4$) into inorganic phosphate. Acid and alkaline phosphatases particularly hydrolyse the ester bonds binding P to C (C-O-P ester bonds) in organic matter. During the process, inorganic P is released from organically bound P such as leaf litter, dead root systems, and other organic debris without concomitant release of C (Harrison, 1983). Phosphatase is concentrated in the surface layer and rhizosphere where most of the fresh and less humified organic matter is prevailing (Tarafdar *et al*., 2001). Phosphatases play a crucial role in the phosphorous acquisition of plants and microorganisms, and thus in the cycling of it within the soil (Schneider *et al.*, 2001).

Information on the nature of urease activity in soil was beneficial to develop and employ strategies for nitrogen management. Urease hydrolysis activity is elevated in aerobic condition, and its hydrolysis varied to the plant growth stage within green manure amendment to the crop (Pattnaik *et al*., 1999). Urease activity is not well when the bioavailability in the soil is troubled Saliha *et al.,* (2006) confirm that urease activity increased along with microbial population in soil amended with liquid organic substrate.

## Materials and Methods

*Site description*: The present investigation was carried out in the on-going AICRP on Integrated Farming Systems which was initiated in *kharif,* 1988 at the College Farm, College of Agriculture, Rajendranagar, Hyderabad. The monthly mean maximum temperatures during the crop growth period ranged from 28.0 $^0$C to 37.7 $^0$C with an average of 31.3 $^0$C, while the monthly mean minimum temperature ranged from 10.1 $^0$C to 23.9 $^0$C with an average of 19.2 $^0$C. The total rainfall received during the crop growth period was 1052.7 mm distributed throughout the year.

*Experimental design and treatments*: This experiment was laid out in randomized block design with eight treatments and three replications. The treatment details during *kharif* season were as follows:

$T_1$ – Control (No fertilizer, no organic manure)
$T_2$ – 100 % RD of NPK
$T_3$ – 50 % RD of NPK + 50 % N through FYM
$T_4$ – 75 % RD of NPK + 25 % N through FYM
$T_5$ – 50 % RD of NPK + 50 % N through Paddy straw
$T_6$ – 75 % RD of NPK + 25 % N through Paddy straw
$T_7$ – 50 % RD of NPK + 50 % N through Green leaf manure
$T_8$ – 75 % RD of NPK + 25 % N through Green leaf manure
The treatments in *rabi* season were as follows:
$T_1$ – Control (No fertilizer, no organic manure)
$T_2$ – 100 % RD of NPK (120-60-60 kg N, $P_2O_5$ and $K_2O$ $ha^{-1}$)
$T_3$ – 100 % RD of NPK
$T_4$ – 75 % RD of NPK
$T_5$ – 100 % RD of NPK
$T_6$ – 75 % RD of NPK
$T_7$ – 100 % RD of NPK
$T_8$ – 75 % RD of NPK

The organic sources such as FYM, Paddy straw and *Glyricidia* (green leaf manure) were applied two weeks before transplanting of paddy as per the treatments. All the PK and 1/3$^{rd}$ of N fertilizer were applied at the time of

transplanting while remaining nitrogen was applied in two equal splits. All cultural practices were performed.

*Soil sample analysis:* The initial soil was sandy clay loam, neutral in reaction pH 8.5 (Jackson, 1973), non saline in nature EC 0.24 dS $m^{-1}$ (Jackson, 1973) (Table 1), medium in organic carbon OC 0.54 % (Walkley and Black (1934), low in available N 151 kg $ha^{-1}$ (Subbiah and Asija (1956), medium in available P 24.0 kg $ha^{-1}$ (Olsen *et al.*, 1954) and medium in available K 224 kg $ha^{-1}$ (Jackson, 1973). Urease activity was assayed by quantifying the rate of release of $NH_4^+$ from the hydrolysis of urea as described by Tabatabai and Bremner (1972).

Dehydrogenase activity in the soil was determined by the procedure given by Casida *et al.,* (1964). The method involved spectrophotometric determination of the Tri Phenyl Formazon (TPF) produced when soil is treated with Triphenyl Tetrazolium Chloride (TTC). The acid and alkaline phosphatase activity was assayed by quantifying the amount of p-nitrophenol released and expressed as µg of p-nitrophenol released $g^{-1}$ soil $h^{-1}$ as described by Tabatabai and Bremner (1969).

*Statistical analysis*: The data on the observations made were analyzed statistically by applying the technique of analysis of variance for randomized block design as suggested by Panse and Sukhatme (1978).

## Results and Discussion

Soil enzyme activity is an indirect indication on the activities of microbes which is directly correlated with soil microbial dynamics. Enzyme activity in the soil environment is considered to be a major contributor of overall soil microbial activity (Burns *et al.*, 2013). Due to the effects of external disturbance on their activity, enzymes can serve as sensitive indicators of soil quality (Dick *et al.,* 1994: Nedunchezhiyan *et al.,* 2013). The data pertaining to the effect of different INM treatments on the enzyme activities and their correlations with organic carbon content were presented in the table 2.

### Urease (µg of $NH_4^+$-N $g^{-1}$ soil $2h^{-1}$)

Urease activity ranged from 32.50 to 55.76 µg of $NH_4^+$ released $g^{-1}$ soil $2h^{-1}$ at harvest of *kharif* rice (Table 4.4). The highest urease activity (µg of $NH_4^+$ released $g^{-1}$ soil $2h^{-1}$) was recorded in the treatment receiving 75 % RD of NPK + 25 % N through FYM ($T_3$) followed by $T_4$ (53.38), $T_7$ (52.25) and $T_8$ (50.63). However, $T_4$, $T_7$ and $T_8$ were on par with $T_3$ but significantly superior to $T_2$ (49.73), $T_5$ (49.63) and $T_6$ (45.88).

Urease activity ranged from 54.23 to 30.14 µg of $NH_4^+$ released $g^{-1}$ soil $2h^{-1}$ at harvest of *rabi* rice. The highest urease activity was recorded in the treatment of 75 % RD of NPK ($T_3$) followed by $T_4$ with urease activity of 53.84 µg of $NH_4^+$ released $g^{-1}$ soil $2h^{-1}$ (Table 2).

Long-term organic amendments increased the capacity of the small-sized fractions to protect soil microorganisms; urease activity was mainly located in that fraction (Kandeler, 1996), and the activities of total urease significantly correlated with microbial biomass-C (Klose and Tabatabai, 1999).

### Dehydrogenase (µg of TPF $g^{-1}$ soil $day^{-1}$)

Dehydrogenase is an enzyme that occurs in all intact viable microbial cells. These soil enzymes function as a measure of the metabolic state of soil microorganisms by relating it to the presence of viable microorganisms and their oxidative capacity.

**Table.1** Long-term effects of INM on available N, P and K status (kg $ha^{-1}$) of the soils after harvest of rice-rice system at Rajendranagar

| Treatments | | OC (%) | | N | | $P_2O_5$ | | $K_2O$ | |
|---|---|---|---|---|---|---|---|---|---|
| ***Kharif*** | ***Rabi*** | ***Kharif*** | ***Rabi*** | ***Kharif*** | ***Rabi*** | ***Kharif*** | ***Rabi*** | ***Kharif*** | ***Rabi*** |
| **$T_1$ – Control** | $T_1$ – Control | 0.57 | 0.59 | 125.5 | 124.7 | 18.6 | 16.2 | 204.5 | 200.1 |
| **$T_2$ – 100 % RD of NPK** | $T_2$ – 100 % RD of NPK | 0.68 | 0.57 | 196.5 | 208.3 | 32.9 | 34.8 | 362.6 | 380.5 |
| **$T_3$ – 50 % RD of NPK + 50 % N through FYM** | $T_3$ – 100 % RD of NPK | 0.72 | 0.65 | 217.4 | 233.1 | 44.0 | 45.5 | 392.9 | 394.0 |
| **$T_4$ – 75 % RD of NPK + 25 % N through FYM** | $T_4$ – 75 % RD of NPK | 0.67 | 0.61 | 230.0 | 216.0 | 40.4 | 40.6 | 363.3 | 355.7 |
| **$T_5$ – 50 % RD of NPK + 50 % N through Paddy straw** | $T_5$ – 100 % RD of NPK | 0.70 | 0.68 | 179.8 | 200.7 | 33.9 | 32.6 | 320.6 | 347.2 |
| **$T_6$ – 75 % RD of NPK + 25 % N through Paddy straw** | $T_6$ – 75 % RD of NPK | 0.71 | 0.70 | 183.1 | 198.2 | 32.3 | 30.3 | 306.4 | 332.6 |
| **$T_7$ – 50 % RD of NPK + 50 % N through Green leaf manure** | $T_7$ – 100 % RD of NPK | 0.68 | 0.60 | 204.0 | 218.3 | 38.6 | 36.2 | 356.5 | 387.7 |
| **$T_8$ – 75 % RD of NPK + 25 % N through Green leaf manure** | $T_8$ – 75 % RD of NPK | 0.66 | 0.71 | 209.1 | 207.3 | 37.0 | 29.8 | 344.5 | 370.0 |
| **Initial** | | 0.54 | | 151.0 | | 24.0 | | 224.0 | |
| **CD (P=0.05)** | | NS | 0.09 | 28.7 | 30.00 | 8.01 | 2.91 | 50.08 | 60.69 |
| **SEm ±** | | 0.04 | 0.03 | 9.70 | 10.23 | 2.67 | 1.03 | 16.65 | 20.47 |

**Table.2** Long-term effects of INM on enzyme activities of the soils under rice-rice cropping system at Rajendranagar

| Treatments | | Acid phosphatase | | Alkaline phosphatase | | Dehydrogenase (µg of TPF $g^{-1}$ soil $day^{-1}$) | | Urease (µg of $NH_4^+$-N $g^{-1}$ soil $2h^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|
| | | (µg p-nitrophenol $g^{-1}$ soil $h^{-1}$) | | | | *Kharif* | *Rabi* | *Kharif* | *Rabi* |
| *Kharif* | *Rabi* | *Kharif* | *Rabi* | *Kharif* | *Rabi* | | | | |
| **$T_1$ – Control** | $T_1$ – Control | 38.55 | 35.60 | 98.22 | 94.51 | 290.06 | 255.79 | 32.50 | 30.14 |
| **$T_2$ – 100 % RD of NPK** | $T_2$ – 100 % RD of NPK | 44.41 | 52.60 | 108.54 | 101.53 | 359.03 | 302.34 | 49.73 | 42.61 |
| **$T_3$ – 50 % RD of NPK + 50 % N through FYM** | $T_3$ – 100 % RD of NPK | 79.28 | 70.50 | 149.70 | 129.53 | 488.19 | 364.25 | 55.76 | 54.23 |
| **$T_4$ – 75 % RD of NPK + 25 % N through FYM** | $T_4$ – 75 % RD of NPK | 77.78 | 68.43 | 142.98 | 123.76 | 477.43 | 358.01 | 53.38 | 53.84 |
| **$T_5$ – 50 % RD of NPK + 50 % N through Paddy straw** | $T_5$ – 100 % RD of NPK | 65.20 | 58.33 | 128.93 | 105.83 | 408.20 | 325.61 | 49.63 | 46.95 |
| **$T_6$ – 75 % RD of NPK + 25 % N through Paddy straw** | $T_6$ – 75 % RD of NPK | 62.73 | 55.56 | 121.10 | 102.63 | 403.06 | 321.18 | 45.88 | 45.08 |
| **$T_7$ – 50 % RD of NPK + 50 % N through Green leaf manure** | $T_7$ – 100 % RD of NPK | 70.86 | 64.40 | 137.26 | 117.73 | 451.15 | 357.23 | 52.25 | 49.76 |
| **$T_8$ – 75 % RD of NPK + 25 % N through Green leaf manure** | $T_8$ – 75 % RD of NPK | 68.52 | 62.46 | 133.47 | 116.65 | 449.28 | 350.00 | 50.63 | 47.98 |
| **CD (P=0.05)** | | 6.93 | 6.27 | 16.39 | 14.69 | 53.67 | 43.31 | 4.05 | 4.97 |
| **SEm±** | | 2.26 | 2.04 | 5.35 | 4.80 | 17.53 | 14.15 | 1.32 | 1.62 |

**Table.3** Simple correlations between different organic carbon and soil enzyme activities

| Variables | | | r-values | |
|---|---|---|---|---|
| | | | *Kharif* | *Rabi* |
| **Organic carbon** | vs | Acid phosphatase | 0.8567 | **0.8800** |
| **Organic carbon** | vs | Alkaline phosphatase | 0.8189 | **0.8203** |
| **Organic carbon** | vs | Dehydrogenase | 0.7455 | **0.7317** |
| **Organic carbon** | **vs** | **Urease** | **0.8233** | **0.8891** |

The results related to dehydrodenase activity revealed that highest activity (488.19 µg of TPF $g^{-1}$ soil $day^{-1}$) was recorded in treatment receiving 50 % RD of NPK + 50 % N through FYM ($T_3$) at harvest of *kharif* crop whereas lowest activity observed in control (290.06 µg of TPF $g^{-1}$ soil $day^{-1}$). However, $T_3$ showed on par results with $T_4$ (477.43), $T_7$ (451.15) and $T_8$ (449.28) and significantly increased dehydrogenase activity (µg of TPF $g^{-1}$ soil $day^{-1}$) than $T_2$ (359.03), $T_5$ (408.20) and $T_6$ (403.06) (Table 2).

Dehydrogenase activity during *rabi* season indicated that highest activity (364.25 µg of TPF $g^{-1}$ soil $day^{-1}$) was observed in treatment 100 % RD of NPK ($T_3$) followed by $T_4$ (100 % RD of NPK) and $T_7$ (100 % RD of NPK) and $T_8$ (75 % RD of NPK) with dehydrogenase activity of 358.01, 357.23 and 350.0 µg of TPF $g^{-1}$ soil $day^{-1}$, respectively, and on par with $T_3$. The lowest activity was observed in (255.79 µg of TPF $g^{-1}$ soil $day^{-1}$) control (Table 2).

Marinari *et al.*, (2000) showed that a higher level of dehydrogenase activity was observed in soil treated with vermicompost and manure compared to soil treated with mineral fertilizer. In this study, the combined effect of organic manure and chemical fertilizer was also better than that of only chemical fertilizers. Because biological energy matter such as organic manure can supply available energy, thus it can accelerate microorganism and enzyme cell multiplication to improve organism and enzyme living environment and then to increase soil organism and enzyme composition and activity (Li *et al.*, 2000).

## Acid and Alkaline Phosphatase (µg p-nitrophenol $g^{-1}$ soil $h^{-1}$)

Phosphatases are a group of enzymes that are capable of catalyzing hydrolysis of esters and anhydrides of phosphoric acid. Tabatabai and Eivazi (1977) reported that air drying of the soils increased the activity of acid phosphatase and phosphodiesterase but decrease the activity of alkaline phosphatase. In soil ecosystems, these enzymes play critical roles in P cycles (Speir and Ross, 1978) as evidence showed that they were correlated to P stress and plant growth. The data on acid phosphatase activity revealed that $T_3$ showed highest 79.28 and 70.50 µg p-nitrophenol released $g^{-1}$ soil $h^{-1}$ at harvest of both *kharif* and *rabi* rice, respectively. The lowest 38.55 and 35.60 µg p-nitrophenol released $g^{-1}$ soil $h^{-1}$ in $T_1$ (control). However, $T_3$ and $T_4$ were on par with each other (Table 2). All the INM treatments showed significant effect on acid phosphatise activity under both *kharif* and *rabi*.

The results related to alkaline phosphatase activity revealed that $T_3$ (50 % RD of NPK + 50 % N through FYM) showed highest activity 149.70 and 129.53 µg p-nitrophenol released $g^{-1}$ soil $h^{-1}$ at harvest of *kharif* and *rabi* crops, respectively. The lowest activity of 98.22 and 94.51 µg p-nitrophenol released $g^{-1}$ soil $h^{-1}$ was observed in $T_1$ (control) at harvest of *kharif* and *rabi* crops, respectively (Table 2). All the treatments did not show any significant effect soil acid phosphatase activity.

The activity of enzymes can be attributed to microbial origin developed during decomposition of organic sources of nutrients. Addition of organic sources acts as good source of carbon and energy to heterotrophs by which their population increased with an increase in enzyme activities. Similar relationship between organic carbon and enzyme activities were reported by Bohme and Bohme (2006) and Rai and Yadav (2011).

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